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Published
With international search report.

(54) Title: GENE AND AMINO ACID SEQUENCES FOR NOVEL TRANSCRIPTION FACTOR

(57) Abstract

The present invention relates to a novel transcription factor, called FREAC 11, which is a member of the forkhead family of transcription factors. The invention also relates to the gene freac 11 encoding said transcription factor. FREAC 11 is expressed in adipose tissue and regulates adipose tissue expressed genes involved in lipid metabolism and adipocyte differentiation. Furthermore, the invention relates to drug and drug target applications of FREAC 11 and freac 11, respectively.

GENE AND AMINO ACID SEQUENCES FOR NOVEL TRANSCRIPTION FACTOR

Technical field

The present invention relates to a novel transcription factor. More precisely, the present invention relates to a transcription factor expressed in adipose tissue and involved in lipid metabolism and/or adipocyte differentiation.

Background of the invention

Triglycerids make up approximately 90% of the dietary fat consumed by an adult on a typical western diet, which is equal to 60-100 gr (Hopfer, 1986). These triglycerids will be processed in a series of enzymatic reactions to release free fatty acids (FFA). FFA will be used as an energy source in muscle tissue or stored as an energy buffer in adipose tissue. The rate limiting step in processing dietary triglycerids to FFA is catalyzed by lipoprotein lipase (LPL) (Brunzell, 1989). Thus, LPL is a key enzyme in providing adipose tissue with FFA, which are reesterified and stored as lipid droplets within the adipocyte. It is therefore reasonable to assume that LPL is critical for developing obesity, a notion underscored by the fact that patients with hyperlipoproteinemia type I (ie. patients with no LPL activity) are typically lean (Holt, 1939).

Furthermore, it is a well established fact that obesity in humans is associated with several diseases such as ischemic heart disease (IHD), noninsulin dependent diabetes mellitus (NIDDM), certain types of cancer and mental disorders.

Substances/methods to decrease fat cell mass in obese individuals with NIDDM are highly desired to restore insulin sensitivity and normalize blood glucose levels.

Another known gene involved in lipid metabolism is the ob gene (obese) gene (Da-Wei Gong et al., 1996). In GenBank entry U43589 the sequence of the ob promoter has been deposited.

Further genes of great importance to regulate adipocyte metabolism and/or differentiation are the peroxisome proliferator-activated receptor genes, such as the ppar gamma 2 gene (Zhu et al., 1995).

Summary of the invention

The present invention relates to a new previously unknown gene that is selectively expressed in adipose tissue stromal cells, adipocytes and preadipocytes. The expression product of the gene has unique ability to regulate genes involved in lipid metabolism and/or adipocyte differentation, such as the lpl gene, ob gene, and ppar gamma 2 gene, respectively. In the present description and claims, genes are designated with lower case letters and gene products are designated with capital letters.

The present invention provides a novel human transcription factor, called FREAC 11, which is selectively expressed in adipose tissue. FREAC 11 is a member of the forkhead family of transcription factors (Weigel et al., supra). The invention relates to freac 11 and FREAC 11 and/or amino acid sequence of freac 11 and FREAC 11, and variants thereof respectively.

For the purposes of the invention, it is essential that the conserved DNA binding domain of FREAC 11 is present, although activities of importance for adipocyte metabolism and/or adipocyte differentiation are expected to reside outside of the 110 amino acid DNA binding region. In FREAC 11 such sites are, for example, sites for protein protein interaction and transcription regulative functions. In freac 11 it is essential that the nucleotide sequence encoding the DNA binding domain is present.

According to the invention FREAC 11 is used for transcriptional regulation of of adipocyte expressed genes, such as the lpl gene, the ob gene and the ppar gamma2-gene. The present inventor has found that all these genes contain potential binding sites for FREAC 11, i.e. at

In another embodiment, FREAC 11 is the drug target. For this purpose the invention relates to, in a sixth aspect, use of FREAC 11 transcription factor for high throughput screening, HTS, for substances influencing the activity of FREAC 11, such as inhibitors, antagonists, agonists etc..

For the purpose of the invention, gene means both genomic DNA, cDNA, and synthetic DNA.

The above described drugs and drug target applications of the invention are intended for use in therapy of obesity-related conditions where it is desired to either increase or decrease the activity of adipocyte expressed genes, such as obesity, non-insulin dependent diabetes mellitus, cardiovascular diseases, catabolic conditions, anorexia, bulemia.

Detailed description of the invention

The invention will now be described more closely below in association with the accompanying drawings and some non-limiting examples.

In the drawings:

Fig. 1 shows nucleotide and amino acid sequence of FREAC 11 wherein the DNA binding domain is underlined;

Fig. 2 shows a Northern blot of RNA from different tissues using a part of the cDNA in fig 1 located outside of the DNA-binding region as a probe;

Fig. 3 shows a diagram of the induction of LPL during adipogenesis measured as reporter gene activity derived from a lpl promoter-reporter gene construct; the filled bars show 3T3-F442A cells transfected with a lpl promoter-reporter gene construct only; and the open bars show a corresponding experiment with a lpl promoter- reporter gene construct that has been transfected into differentiating 3T3-F442A adipocytes together with a construct expressing only the DNA binding region of freac 11.

present in the closed bar experiment, see legend to Fig. 3. Expression of S12 box (open bars) inhibits the normal LPL induction (filled bars).

In this way it is possible to study the effects of the endogenously expressed FREAC-11 in these cells by blocking its access to the lpl promoter.

The transcription factor FREAC 11 according to the invention is exclusively expressed in adipose tissue when the DNA binding domain of this transcription factor is expressed in stable transfectants. It will act as a negative dominant mutation and LPL levels, measured as reporter gene activity, are down to base line values, see Fig. 3. Thus, the DNA binding domain of FREAC 11 will bind to the two cis elements in the LPL promoter- in doing so this cis elements will be blocked and not able to interact with wild type transcription factors.

EXAMPLE 3: Transfected freac 11 inhibits ob promoter activity

To study the regulative properties of FREAC 11 a co-transfection was performed as depicted in Fig. 4. This shows that when an expression vector encoding the DNA binding region (= S12 box) of freac 11 is transfected together with a ob promoter-reporter gene a clear reduction in reporter gene activity is evident. This implicates FREAC-11 not only as a regulator of lpl gene but also as a regulator of the ob gene. This experiment provides evidence that the ob promoter contains functional cis-elements capable of interacting with the DNA binding domain of FREAC 11. Both freac 11 and the ob-gene are selectively expressed in adipose tissue.

EXAMPLE 4.Body weight of trangenic mice

The body weight of control, transgenic mice with 1-3 extra copies of FREAC 11 (low copy transgenic) in the genom and transgenic mice having more than five extra copies of FREAC 11 in the genom 11 (high copy transgenic) was compared in 73 animals.
It was found that the body weight is significantly elevated in high copy transgenic mice in comparison to low copy transgenic and control, both for male and female.

Through the use of yeast 2- hybrid screening systems, or similar systems, possible protein - protein interactions will be investigated - such proteins ie proteins that interact with FREAC-11 could also be used to modulate the activity of FREAC-11 and in doing so also regulating LPL and adipocyte differentiation /metabolism.

Besides proteins, the interaction of FREAC 11 with other molecules than proteins, such as carbohydrats, lipids, and other compounds, will also be investigated.

EXAMPLE 6. The freac-11gene as a drug target

For this purpose an antisense construct of freac 11 will be produced. If freac 11 is inhibited in this way, an increased activity is expected of adipocyte expressed genes containing the described cis elements.

References

Hopfer, U. (1986) *Biochemistry* (Devlin, T. ed. second edition), 931-938, John Wiley & Sons, New York.

Brunzell, J.D. (1989) *The Metabolic Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L. and Valle, D. eds.), 1165-1180, McGraw-Hill, New York.

Holt, L.E., Jr.; Aylward, F.X.; Timbers, H.G.: Idiopathic familial lipemia. *Bull. Johns Hopkin Hosp.* 64; 279-314.

Kern P.A., (1995) The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase in *J Clin Invest* 5; 2111-2119.

CLAIMS

1. Freac 11 gene (cDNA or genomic DNA) comprising the nucleotide sequence of Fig. 1 as well as fragments and allelic variants thereof encoding transcription regulative products.

2. Freac 11 sequence according to claim 1 comprising the underlined nucleotide sequence in Fig. 1.

3. Freac 11 sequence according to claims 1 or 2, which encodes a product having transcription regulative function directed against adipose tissue expressed genes.

4. FREAC 11 transcription factor comprising the amino acid sequence of Fig. 1 as well as fragments and variants thereof having transcription regulative function.

5. FREAC 11 transcription factor according to claim 4 comprising the underlined amino acid sequence in Fig. 1.

6. FREAC-11 transcription factor according to claims 4 or 5, wherein the transcription regulative function is directed against adipose tissue expressed genes.

7. FREAC 11 transcription factor according to claims 4-6, wherein FREAC 11 is involved in lipid metabolism and/or adipocyte differentation.

17. Use of FREAC 11 transcription factor according to any one of claims 4-9, for high throughput screening, HTS, for substances influencing the activity of FREAC 11.

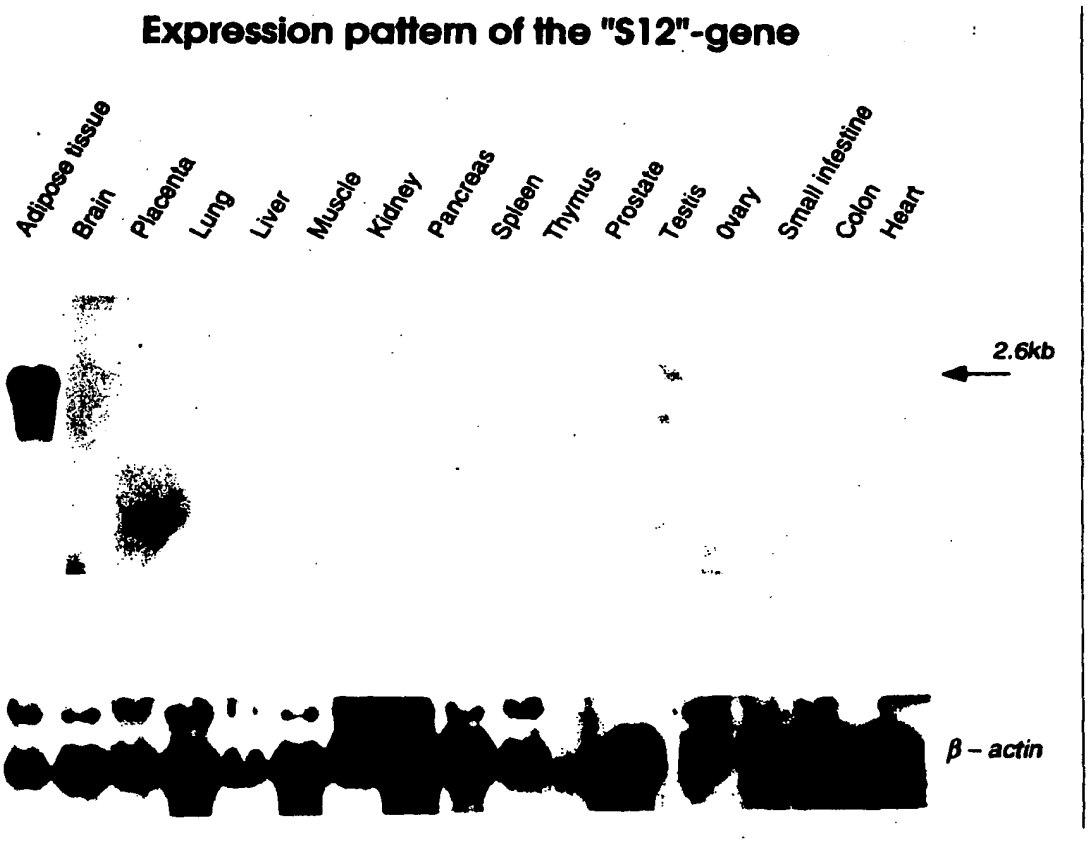
Expression pattern of the "S12"-gene
Adipose tissue
Brain
Placenta
Lung
Liver
Muscle
Kidney
Pancreas
Spleen
Thymus
Prostate
Testis
Ovary
Small intestine
Colon
Heart
2.6kb
β – actin

Fig. 2

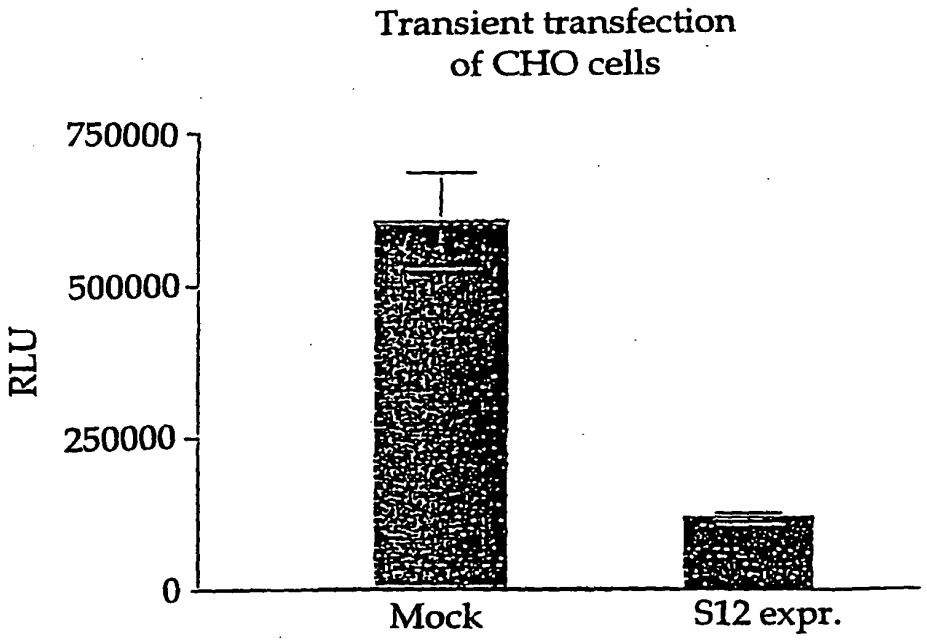
Transient transfection
of CHO cells
750000
500000
250000
0
RLU
Mock
S12 expr.
Reporter:
human 6,5 kb Ob promoter-luciferase

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00989

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/435, C07K 14/47, A61K 38/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, DBA, SCISEARCH, WPI, US PATENTS FULLTEXT, EMBL/PIR /SWISSPROT/GENESEQ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Genomics, Volume 41, 1997, Naoyuki Miura et al, "Isolation of the Mouse (MFH-1) andHuman (FKHL14) Mesenchyme Fork Head-1 Genes Reveals Conservation of Their Gene and Protein Structures", page 489 - page 492, See Figure 1	1-9
Y		17
	--	
X	FEBS Letters, Volume 326, No 1,2,3, July 1993, Naoyuki Miura et al, "MFH-1, a new member of the fork head domain family, is expressed in developing mesenchyme" page 171 - page 176	1,4
Y		17
A		2-3,5-16
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 Sept 1998	17 -09- 1998
Name and mailing address of the ISA/ Swedish Pat nt Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application N .

PCT/SE 98/00989

Box I Observations where certain claims were found unsearchable (Continuati n of item 1 f first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) f r the following reasons:

1. [x] Claims Nos.: 11-12 and 14-15
because they relate to subject matter not required to be searched by this Authority, namely:

With the present wording claims 11-12 and 14-15 are directed to a method of treatment of the human/animal body. However, the search has been carried out, based on the alleged effects of the compound (c.f. PCT Rule 39.1(iv))

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL-TYPE SEARCH REPORT

(PCT Article 15.5)

National application No.	Country or Office of filing	Applicant's or agent's file reference
0003435-5	SE	00298-SE

Filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
26 September 2000	

Applicant

Pharmacia & Upjohn AB

Date of request for international-type search	International-type search request No.
26 September 2000	SE 00/01156

This international-type search report has been prepared by this International Searching Authority and is transmitted to the applicant.

This international-type search report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [X] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international-type search was carried out on the basis of the sequence listing

   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

INTERNATIONAL-TYPE SEARCH REPORT

Search request No.
SE00/01156

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international-type search report has not been established in respect of certain claims for the following reasons:

1. ☐ Claims No.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims No.:
because they relate to parts of the national application that do not comply with the prescribed requirements to such an extent that no meaningful international-type search can be carried out, specifically:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this application, as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international-type search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international-type search report covers only those claims for which fees were paid, specifically claims No.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international-type search report is restricted to the invention first mentioned in the claims, it is covered by claims No.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of:

The invention according to claims 1-4 is consequently not considered to involve an inventive step.

The method for diagnosing transmissible spongiform encephalopathy (TSE) in a mammal, according to claims 5-8, is only considered here as far as it concerns an *in vitro* method for diagnosing TSE (see box III). The fact that both the normal and the abnormal forms of the prion proteins are determined enables the calculation of the percentage of abnormal PrP. The findings that TSE-positive animals have a percentage of abnormal PrP higher than 10 % is a way of interpreting the results of the method. The description does not disclose any unexpected advantages with this procedure, and that using the quotient instead of the absolute amount of abnormal PrP reduces the sensitivity of the method to protein concentration, is considered obvious to a person skilled in the art. Therefore, claims 5-8 are not considered to involve an inventive step.

Consequently, the invention according to claims 1-8 fulfils the requirements of novelty and industrial applicability, but does not fulfil the requirement of an inventive step.

INTERNATIONAL-TYPE SEARCH REPORT

Search request No.
SE 00/01156

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/87, C07K 14/47, C12Q 1/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N, C07K, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GENOMICS, Volume 41, 1997, Naoyuki Miura et al, "Isolation of the Mouse (MFH-1) and Human (FKHL14) Mesenchyme Fork Head-1 Genes Reveals Conservation of Their Gene and Protein Structures", page 489 - page 492, & EMBL Accession No.Q99958 and Y08223 have more than 95% identity ---	1-33
X	WO 9854216 A1 (PHARMACIA & UPJOHN AB), 3 December 1998 (03.12.98), & EMBL Accession no. AAY01097 and AAX28103 have more than 98% identity ---	1-33

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international-type search	Date of mailing of the international-type search report
5 July 2001	2001 -12- 27
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson/BS Telephone No. +46 8 782 25 00

INTERNATIONAL-TYPE SEARCH REPORT

Search request No.
SE 00/01156

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Genome Research, Volume 7, No 10, 1997, Ross C. Hardison et al, "Long Human-Mouse Sequence Alignments Reveal Novel Regulatory Elements: A Reason to Sequence the Mouse Genome", page 959 - page 966, see page 959, column 2, line 36 - page 960, column 1, line 17	1-9,12-19
	--	
A	Database EMBL, Genbank/DDBJ, accession no. 009108, DOE Joint Genome Institute: "Sequencing of Human Chromosome 16", 1999-08-04	1-24
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INTERNATIONAL-TYPE SEARCH REPORT

Information on patent family members

06/11/01

Search request No.

SE 00/01156

WO	9854216	A1	03/12/98	AU	7682698	A	30/12/98
				AU	7875698	A	17/07/98
				EP	0946914	A	06/10/99
				EP	1003776	A	31/05/00
				JP	2001507149	T	29/05/01
				NO	995786	A	25/01/00
				SE	9701963	D	00/00/00